Clinical review

Management of pregnancies with RhD alloimmunisation

Sailesh Kumar, Fiona Regan

Pregnancies complicated by red cell alloimmunisation may result in fetal anaemia secondary to transplacental passage of maternal immunoglobulin G, which causes progressive fetal haemolysis. In severe cases the anaemic fetus develops ascites, subcutaneous oedema, and pleural and pericardial effusions (hydrops fetalis) and dies in the womb. Many different antibodies (anti-D, anti-Kell, anti-c, anti-E, etc) can cause haemolytic disease of the fetus and newborn. This review covers the management of pregnancies affected with RhD alloimmunisation. The principles of management are similar regardless of the type of antibody involved, although care needs to be taken with pregnancies complicated by Kell alloimmunisation, where antibody concentrations do not always correlate with disease severity.

We searched PubMed for up to date references on current advances in the treatment of RhD alloimmunisation. In addition, we used guidelines on antenatal care and prophylaxis from the websites of the National Institute for Clinical Excellence (NICE, www.nice.org.uk) and the Royal College of Obstetricians and Gynaecologists (www.rcog.org.uk).

Hydrops fetalis was first described in 1609 by a French midwife, Louise Borgeois,[1] after the birth of twins one of whom was oedematous and the other deeply jaundiced; both died soon after the birth. However, it was not until 1940 that Landsteiner and Weiner, using rhesus monkeys, discovered the RhD antigen.[2]

Summary points

- The incidence of RhD alloimmunisation is decreasing
- Genotyping of fetuses can now be done non-invasively, using maternal plasma
- Serial amniocentesis to assess progression of fetal anaemia is no longer necessary
- Fetal anaemia can be monitored non-invasively by using Doppler ultrasonography of the middle cerebral artery
- Anaemia in late infancy may require top-up transfusions or erythropoietin, or both
- Good neurodevelopmental outcome can be expected in treated fetuses
- Immunotherapy may be of benefit in selected cases

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BMJ 2005;330:1255–8

Pathophysiology

The RhD polypeptide is an integral membrane protein expressed exclusively on erythrocytes. Some 16% of white people are RhD negative because of deletion of the gene. During pregnancy, small volumes of fetal red cells continually get into the mother's circulation. This trafficking of red cells increases as gestation progresses. Bowman et al showed at least 0.01 ml of fetal cells in 3%, 12%, and 46% of women in each trimester.[3] In most women, this load of RhD antigen on fetal erythrocytes and erythrocyte precursors does not stimulate the mother's immune system because fetal red cells are rapidly cleared by her reticulo-endothelial system. However, when a large volume of fetal blood enters the mother's circulation, her immune system is stimulated and B lymphocyte clones that recognise the RhD antigen are established. The initial IgM anti-D immunoglobulin response is short lived, with a rapid switch to IgG production. Unlike IgM, IgG anti-D crosses the placenta and destroys fetal erythrocytes, causing fetal anaemia. Haemolytic disease of the newborn can range in severity from being detectable only in laboratory tests through to severe fetal anaemia resulting in hydrops, stillbirth, or the birth of babies with severe anaemia and jaundice. Fortunately kernicterus, a devastating condition secondary to bilirubin deposition in the brain stem, has become rare ever since hyperbilirubinaemia in newborns has been treated aggressively. In England and Wales, about 500 fetuses develop haemolytic disease each year, and about 25-30 babies die from haemolytic disease of the newborn.

Immunoprophylaxis

Before immunoprophylaxis became available, haemolytic disease of the newborn affected 1% of all newborns and was responsible for the death of one baby in every 2200 births. Antenatal and postnatal administration of anti-D immunoglobulin is now clearly established to prevent RhD alloimmunisation. However, for it to work it must be given in sufficient dose and before immunisation has occurred. The mechanism by which it exerts its effect is unknown. It

may work by causing a negative feedback mechanism or by blocking RhD antigenic determinants on the red cell membrane, among other postulated mechanisms. The most important cause of anti-D antibodies now is immunisation during pregnancy, when no overt sensitising event has occurred. Late immunisation during a first pregnancy is responsible for 18-27% of cases. Immunisation during a second or subsequent pregnancy probably accounts for a similar proportion of cases, although it is often impossible to distinguish late sensitisation from failure of prophylaxis at the end of the preceding pregnancy.

Current recommendations for immunoprophylaxis from the Royal College of Obstetricians and Gynaecologists and NICE are as follows:[4 5]

- After delivery, irrespective of the dose of antenatally administered anti-D immunoglobulin, postnatal prophylaxis must be given and include a screening test to identify women with a large fetomaternal haemorrhage who need additional immunoglobulin
- Anti-D immunoglobulin should be given after sensitising events before delivery and after abortion
- Anti-D immunoglobulin is no longer necessary in women with threatened miscarriage with a viable fetus and cessation of bleeding before 12 weeks' gestation
- At least 500 IU of anti-D immunoglobulin should be given to non-sensitised RhD negative women at 28 weeks and 34 weeks of pregnancy.

Sensitising events include threatened miscarriage, ectopic pregnancy, any invasive prenatal procedure (chorionic villous sampling, amniocentesis, etc), antepartum haemorrhage, external cephalic version, closed abdominal injury, and intrauterine injury.

Patients who are weakly RhD positive (previously Du positive) have a quantitative rather than a qualitative difference in the D antigen and are not at risk of RhD alloimmunisation. They therefore do not require prophylaxis with anti-D immunoglobulin.

Management

A detailed history for the cause of the alloimmunisation (inadequate prophylaxis, administrative failure, blood transfusion, etc) should be taken. Details of previously affected pregnancies—particularly transfusions in the womb, neonatal anaemia, and the need for exchange transfusions or phototherapy—should also be obtained. This information enables a risk assessment of the pregnancy. The paternal genotype should be ascertained. If heterozygous, there is a 50% chance that the fetus is RhD positive and therefore at risk.

Monitoring

Once alloimmunisation has occurred the fetus is at risk from anaemia, and this risk seems to increase with increasing concentrations. In the United Kingdom and Europe a threshold value of 15 IU/ml has been recommended for invasive testing as only mild haemolytic disease is noted with anti-D levels below this value.[6] Maternal anti-D concentrations should be checked every four weeks. At Queen Charlotte's and Chelsea Hospital, patients are referred to the fetal medicine unit once this measurement exceeds 4 IU/ml as we have had cases of severe fetal anaemia even at such relatively low concentrations. However, in many other institutions the threshold for referral remains at 15 IU/ml.[6] If paternal testing indicates heterozygosity, the fetal genotype should be ascertained.

The management of RhD alloimmunisation has been revolutionised by two important discoveries. Firstly, it is now possible to establish fetal Rh genotype non-invasively by using a maternal blood sample. Using polymerase chain reaction techniques, fetal RhD status can be detected with 100% sensitivity.[7 8] Fetal DNA extracted from maternal plasma is analysed for the RhD gene with a fluorescence based polymerase chain reaction test that is sensitive enough to detect the RhD gene in a single cell. This technique has obviated the need for invasive fetal testing to ascertain the fetal genotype. The fetal Kell and c genotype can now also be ascertained by using this non-invasive method. The second advance in management is the use of velocimetry of the fetal middle cerebral artery (fig 1) to monitor affected pregnancies. Peak systolic velocities greater than 1.5 multiples of the median for the specific gestation are predictive of moderate or severe fetal anaemia,[9] with 100% sensitivity and a false positive rate of 12%. However, this method of monitoring should be used with caution after 36 weeks. Pregnancies at risk should be monitored on a weekly basis. Doppler ultrasonography of the middle cerebral artery correlates well with increasing levels of bilirubin in the amniotic fluid.[10] This non-invasive method has superseded the traditional technique of serial amniocentesis for the spectral analysis of amniotic fluid at 450 nm (ΔOD_{450}) first described by Liley in 1961.[11] Liley's technique was used to measure bilirubin in amniotic fluid, an indirect measure of fetal haemolysis.

Fetal blood sampling and intrauterine transfusion

If monitoring of the middle cerebral artery indicates anaemia, fetal blood sampling and intrauterine transfusion are indicated. The patient should be counselled that the loss rate related to the procedure depends on the gestation, site of sampling, and underlying pathology. The risk of uncomplicated fetal blood sampling is 1-3%, but if the fetus is hydropic it may be as high as 20%.[12] Ideally intervention should be delayed until at

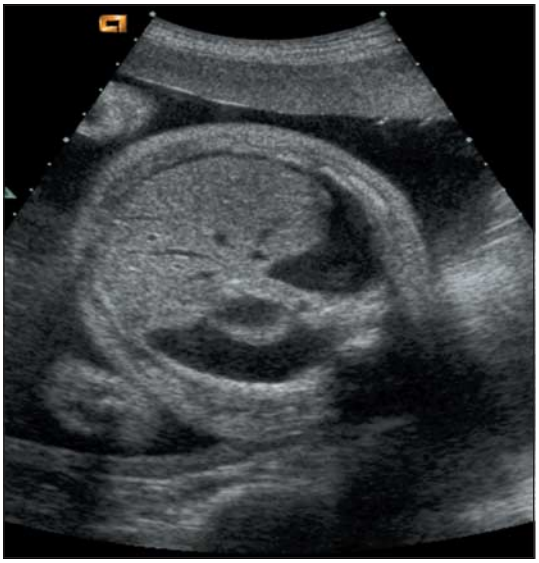

Fig 1 Circle of Willis in the fetus with the cursor placed on the middle cerebral artery and the Doppler wave form

Fetal blood sampling and intrauterine transfusion: key points

- Blood can be sampled from the placental cord insertion site or the intrahepatic vein
- The risk of fetal loss as high as 20% depending on the condition of the fetus
- The procedure should be performed in fetal medicine units
- The fetus's condition should be monitored with Doppler ultrasonography of the middle cerebral artery between transfusions

least 18 weeks' gestation, although it may be technically possible to sample fetal blood from as early as 16-17 weeks.

Fetal blood can be taken from either the placental cord insertion site or the intrahepatic vein (fig 2). Of the two methods, the intrahepatic approach is less likely to cause fetal distress but is technically more challenging.

Complications of fetal blood sampling include fetal bradycardia, haemorrhage, cord haematoma and tamponade, and fetal death. The procedure is done under continuous ultrasound guidance, and facilities for immediate analysis of the fetal blood should be available. Group O negative, cytomegalovirus negative blood that has been cross matched with a maternal blood sample is used for fetal transfusion. Typically the donor cells are packed to a volume of 75-90% to prevent volume overload, and they are irradiated to minimise the risk of graft versus host disease. A final fetal packed cell volume of 55-60% is desirable after the transfusion.

Although the timing of subsequent transfusions is dependent on the rate of decline of the fetal packed cell volume, the presence of hydrops, and gestation, an interval of three to five weeks is the norm. Close monitoring with Doppler ultrasonography of the middle cerebral artery is essential.

Timing of delivery

With careful monitoring and appropriate timing of transfusions, delivery should be anticipated at 37-38 weeks' gestation.[13] If complications occur during an intrauterine transfusion after 32 weeks immediate delivery should be considered. Antenatal steroids for lung maturity may be considered if preterm delivery is anticipated. At delivery, cord blood should be collected for analyses of haemoglobin, packed cell volume, and bilirubin, and for a direct antiglobulin test (DAT). The mode of delivery is dependent on standard obstetric grounds. Prior intrauterine therapy is not an indication for an elective caesarean section.

Outcome

Survival rates of fetuses with anaemia have improved considerably since intrauterine transfusion was introduced. Nevertheless, the reported survival rates of fetuses with and without hydrops and when fetal anaemia presents very early in gestation are still drastically different.[14 15] Reversal of hydrops as a result of intrauterine treatment is associated with improved perinatal outcome.[16] In cases where the hydrops did not reverse the survival rate was only 39%.[16] The irreversibility of a proportion of cases despite successful correction of fetal anaemia remains an enigma. One hypothesis is the development of severe injury to the fetal endothelium[17] as a consequence of iron overload, which results from intrafetal haemolysis.[18] In one review the overall survival was noted to be 84% with non-hydropic fetuses having better outcomes (92%) than hydropic fetuses (70%).[19]

Neonatal anaemia

Newborns may experience ongoing anaemia. Early anaemia is usually the result of passively acquired maternal antibodies causing ongoing haemolysis. The criteria for performing exchange transfusions remain controversial,[20] but rapidly rising serum concentrations of bilirubin that are unresponsive to intensive phototherapy are an indication. The most common cause of late anaemia is a hyporegenerative anaemia, usually after several intrauterine transfusions. Affected infants have suppression of erythropoiesis with extremely low reticulocytes despite a low packed cell volume and normal erythropoietin values. Bone marrow aspirates show erythroid hypoplasia.[21 22] The infant usually needs top-up transfusions only if symptomatic.[23] Several groups of researchers have shown a decrease in the need for late "top-up" transfusions if the infant is treated with recombinant erythropoietin.[24]

Neurodevelopmental outcome

Normal neurodevelopmental outcome can be expected in more than 90% of cases. With appropriate management, the historical sequela of kernicterus is fortunately seen rarely. Sensorineural hearing loss is more common in infants affected by haemolytic disease of the newborn because of the toxic effect of prolonged exposure of bilirubin on the developing eighth cranial nerve.[25] Other more complex problems are also implicated. A recent meta-analysis[26] showed an overall significant association (odds ratio 2.0) between maternal-fetal RhD incompatibility and schizophrenia.

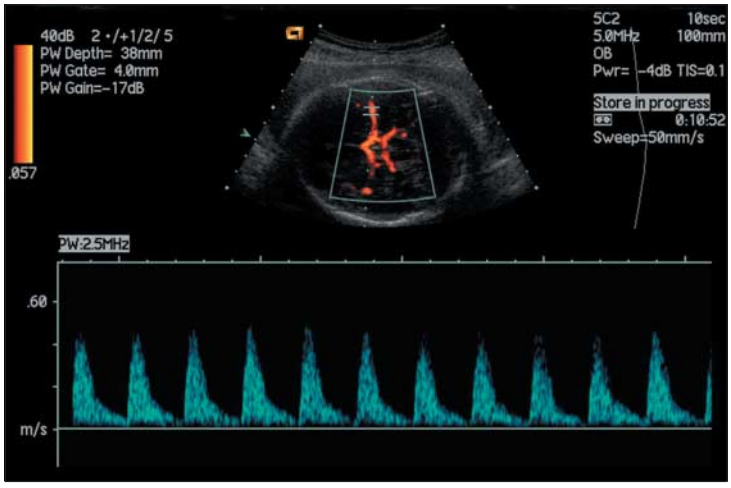

Fig 2 Fetal blood sampling with the needle in the intrahepatic vein

Other treatment modalities

Recent studies using maternal intravenous immunoglobulin have shown some benefit in severe cases of RhD incompatibility.[27 28] The mechanism of action is still not well understood but may entail downregulation of the maternal immune response, placental antigenic blockade, or antigenic blockade at the level of the fetal reticulo-endothelial system. Whatever the mechanism, it is not uniformly effective in inhibiting haemolysis, but when it does, serum bilirubin concentrations fall. This treatment modality is appropriate only in selected cases—for example, very early disease. It may prolong the time interval before the first intrauterine treatment is required. Immunisation to paternal leucocytes in an animal model has been described and has been shown to prevent haemolytic disease.[29] This technique, although showing promise, is still not yet in clinical use.

Conclusions

Although the incidence of haemolytic disease of the newborn has decreased and is no longer a major cause of perinatal mortality, vigilance is still required. Far fewer cases mean less available experience to manage such complicated pregnancies. A strong argument exists for centralising the management of these cases in a few fetal medicine centres that perform enough invasive procedures to maintain skills. Immune therapy in established cases of alloimmunisation show promise but has yet to be translated into routine clinical management.

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(Accepted 13 April 2005)

Corrections and clarifications

Principles for international registration of protocol information and results from human trials of health related interventions: Ottawa statement (part 1)

A wrong URL in the penultimate paragraph of this Education and Debate article by Karmela Krleža-Jerić and colleagues persisted to publication (*BMJ* 2005;330:956-8, 23 Apr). Anyone wishing to contribute to the Ottawa statement on trial registration can do so via http://ottawagroup.ohri.ca. However, although the URL is wrong in the bmj.com version of this article (as well as in the printed journal), the hyperlink does connect to the correct website.

Two drug firms advertised to patients

We read our *British National Formulary* too quickly when checking the generic name for Seretide in this News article by Zosia Kmietowicz (*BMJ* 2005;330:805, 9 Apr). Seretide (GlaxoSmithKline) contains not only fluticasone propionate (as we said) but also salmeterol xinafoate.

FDA warns about using antipsychotic drugs for dementia

Again we got some drug details wrong. In this News article by Jeanne Lenzer (*BMJ* 2005;330:922-3, 23 Apr) we said that Symbyax (Lilly) contained only olanzapine, whereas in fact it also contains fluoxetine hydrochloride.

National survey of UK emergency endoscopy units

We took someone else's error and made it ours in the "What is already known on this topic" box in Andrew Douglass and colleagues' survey (*BMJ* 2005:1000-1, 30 Apr). Endoscopy was done too late in 7% of cases (not 79%, as we stated). The incorrect percentage came from the scoping report of the National Confidential Enquiry into Patient Outcome and Death.